

INTERSEXES IN *PLANTAGO LANCEOLATA*

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(WITH PLATES XII, XIII)

Through the recent researches of GOLDSCHMIDT (11, 12, 13), BANTA (1), WHITMAN, RIDDLE, and their associates (see especially summaries by RIDDLE 22, 23), and LILLIE (17, 18), the facts of intersexualism have acquired a significance which must be considered by any theory of sexuality and sex determination. These new studies show that in widely separated groups of animals which are usually dioecious various grades and degrees of maleness and femaleness in a single individual are common. Judged as entire individuals, such "intersexes" or "sex intergrades" may be predominately male or female, or there may be various grades in the relative development of maleness and femaleness, giving in some cases at least functional hermaphrodites. Along with these there may be individuals that are only male or female. An individual sex organ may start development as of one sex and change to the other, or there may be a decidedly simultaneous development of male and female sex organs, as in the fully functional hermaphrodite. The more remote secondary sex characters also exhibit characteristics of maleness, femaleness, or various grades of modifications that are intermediate.

Such development of intersexuality in forms usually considered as dioecious is evidence that even in dioecious forms sex is not necessarily determined at fertilization, and that sex is not alternative and irreversible for an individual or even for a sex organ. The data are particularly suggestive of the probability that sex differentiation in dioecious forms and in hermaphrodite forms is essentially the same process, and thus that sex determination is on the same fundamental basis in both plants (which are prevailingly hermaphrodite) and animals (which are prevailingly dioecious).

In plants the most intimate association of the two sporophytic sex organs is seen in the so-called perfect flowers. The opposite

extreme is seen in dioecious species. Various grades of sexuality intermediate between these two are seen in species classed as monoecious and polygamous, most striking of which are the numerous instances where all grades of sexuality are to be seen among the various flowers produced by a single individual. DARWIN (7) presents an excellent summary of these cases as evidence that "various hermaphrodite plants have become or are becoming dioecious by many and exceedingly small steps" (p. 181). DARWIN was not directly concerned with the problem of sex determination. He was seeking to discover methods and principles of evolution. In his discussion of sex heteromorphism he places much emphasis on the law of compensation in the utilization of the energy at the disposal of plants, and thus gives recognition to a metabolic theory of sex determination in so far as it relates to the development of the floral organs.

There has been no special dispute over the very obvious fact that the condition of hermaphroditism indicates that sex differentiation may arise through somatic differentiation. According to the sex chromosome theory, however, sex in dioecious species is assumed to be determined qualitatively in reduction divisions and in fertilization, and that the two sexes are hence alternative and represent fundamentally irreversible conditions. In developing this theory, however, little attention has been paid to hermaphrodites, and in view of their predominance in plants the theory cannot be regarded as expressing any broad biological law.

The recent investigations of GOLDSCHMIDT, BANTA, RIDDLE, and LILLIE show that the sex of dioecious species is not necessarily irreversible. This is especially striking, as to demonstration in pedigreed cultures, in the results obtained by BANTA. By means of parthenogenetic reproduction he propagated races from females of *Simocephalus vetulus* for 130 generations, getting nothing but female individuals, only to have the femaleness break up in the 131st generation, giving males, females, hermaphrodites, and many grades of intersexes.

Turning to plants, we have such striking cases of changes of sex combined with conditions of intersexuality as are recently reported by DAVEY and GIBSON (8). They have studied the sex

of the bog myrtle or sweet gale (*Myrica Gale*). This plant is a small shrub which grows abundantly in swamps and heaths in Europe, Asia, and the northern part of North America. The species is described as strictly dioecious, and until 1901 no observations that it is ever otherwise have apparently been recorded. DAVEY and GIBSON find that in the peat moors of England there are everywhere present intersexes, or, as they call them, "mixed plants" of many gradations. Judged as a whole, the plants present every gradation of intersexes between dioecism, monoecism, and hermaphroditism. The variations seen in the various catkins on a single plant include the entire range, and all the grades may appear among the flowers of a single catkin. Furthermore, a study of individual plants for a series of years shows that changes of sex from year to year occur. Plants entirely female in 1913 were entirely male in 1914. Plants female in 1913 were mixed in 1914, entirely or nearly all male in 1915, and again female in 1916.

DAVEY and GIBSON point out that the changes in sex seen in *Myrica Gale* indicate that sex determination is here in some way associated with environmental conditions. In regard to this they state as follows:

The conditions which naturally suggest themselves are moisture, temperature, and light (with their influence on nutrition), and also the previous state of a plant as regards the production of fruit. Since the staminate flowers are developed early in the season preceding that in which they flower, while the pistillate catkins develop much later, it is possible that conditions accelerating or retarding the development of catkin buds may influence the proportions of the two kinds (pp. 150-151).

The facts reported for *Myrica Gale* are striking and suggest that similar conditions may already be present or may spontaneously arise in other species now considered as dioecious.

Intersexes in *Plantago lanceolata*

This species is a native of Europe and Asia. It has been introduced into America, where it has spread from the Atlantic to the Pacific, through Canada, and southward in the United States to Florida. It is well known as a vigorously growing species which in many sections has become a troublesome weed.

Standard botanical treatments describe this species as having only perfect flowers. For over 50 years, however, sex polymorphism has been recognized as present in the species. The tendency has been to group the individuals in 3 classes (LUDWIG 19), most recently designated by BARTLETT (2) as (1) first form hermaphrodite, (2) second form hermaphrodite, and (3) female. CORRENS (4), however, groups plants of this species grown from seed collected near Leipzig, Germany, into 5 classes, in two of which there was variation in single spikes (*a*) from hermaphrodite flowers to flowers with imperfectly developed stamens ($\neq \text{♂}$), and (*b*) from more or less perfect flowers to flowers only female ($\neq \text{♂}$ and ♀). In thus making these classes recognition is given by CORRENS to variations in sex organs which include various grades of gynomonocism already observed in this species by SCHULZ (24).

The difficulty of making any adequate classification, expressed in some degree by CORRENS (4) and by BARTLETT (3), has been very apparent from the observations which the writer has made. In 1912 BARTLETT very kindly supplied me with plants which he classed under the 3 forms just noted. Seed progenies have been grown and observations made of plants growing wild in the fields in and about the New York Botanical Garden, where *P. lanceolata* is exceedingly abundant. Study of this material reveals that there is present a wide range of variations in the development of sporophytic sex organs, which in its general aspects is quite identical with the phenomenon of intersexualism especially described by GOLDSCHMIDT, BANTA, and by DAVEY and GIBSON.

DESCRIPTION OF THE THREE FORMS

Flowers typical of the forms most generally recognized may first be described, as illustrating the two extremes and one intermediate. The flower drawn for a plant was in all cases selected from the middle portion of a spike, and was typical of a large number of flowers in bloom. The flower was placed on a glass slide, a large cover glass was placed over it to bring the various parts into somewhat the same plane, measurement was made of the flower parts under very low magnification by ocular microm-

eter, and all parts were then drawn to scale. Stamens and spores were measured and drawn under higher magnifications.

FIRST FORM HERMAPHRODITE (figs. 1-3, 49, 50).—This term has been applied by BARTLETT (2) to plants whose flowers very uniformly show most complete development of stamens. The filaments are usually twice as long as the pistils. The anthers are large and well developed and white in color. In face view when freshly dehiscent (fig. 2) they measure about 2 mm. in length by 1.5 mm. in width. The corolla lobes are well developed, with blades strongly reflexed when anthesis is complete.

The pollen of numerous plants of this form was examined microscopically and rather extensive germination tests were made.¹ Perfect grains are almost spherical, with thin smooth walls and granular contents. There is much variation in the size of grains that appear to be perfect, the smallest being about one-third the diameter of the largest. There is also a considerable number of obviously imperfect grains with shrunken shriveled walls that are either empty or have hyaline contents. Such grains do not swell up when placed in water or in various media used in testing germination. Impotent grains frequently constitute 25 per cent of the pollen of a microscopical mount. They have always been found present to some extent.

After a rather extended series of experiments it was found that the pollen of this form germinates well in sugar-agar media. The most uniformly favorable results were obtained with a medium of 15 per cent sugar to which 3 per cent agar was added. Good germination was also obtained in 15-1 and 15-5 solutions. The largest tubes observed measured 3.1 mm. in length. Even in the case of the most complete germination not all the spores with granular contents germinated. No shriveled and hyaline spores germinated, but some of the smallest of the apparently perfect spores germinated. Some granular spores of all the sizes failed to germinate. A series of countings was made for a plant

¹ In the various studies of the germinations of pollen reported in this paper the writer has been assisted by Lieut. M. V. REED, a former student and scholar at the New York Botanical Garden, and by Miss HELENE M. BOAS, laboratory assistant, for whose efficient aid and cooperation acknowledgment is here made.

whose pollen germinated most completely. Of 1003 pollen grains placed in 15-1 and 15-3 sugar-agar media, 147 grains (about 15 per cent) had failed to germinate at the end of 24 hours, and of these about half were shrunken and hyaline.

Data regarding the ability to produce seed are of interest in bearing on the condition of femaleness in intersexes. This is a point of particular interest in respect to plants classed as hermaphrodites. LUDWIG (19) reports that the reduction in stamens seen in female plants of *Plantago* is associated with increased fruitfulness. I have made special observations on 3 plants of the first form. In 1916 all of these failed to set any seed to controlled self-pollination. In 1917 two of these failed to produce seed to free open pollination; the third plant was isolated with a pistillate plant. Day after day pollen of the hermaphrodite was very generously shaken over stigmas of both plants. The female plant produced an abundance of seed, while not a seed developed on the other. It is possible that physiological self- and cross-incompatibilities may be operating here (STOUT 25), but the various grades of impotence and intersexuality seen in stamens of plants of this species suggest that the failure to set seed when pollinated with viable pollen may involve impotence of pistils. It is readily observed in the field that many plants fail completely to set seed; although pistils are present they may be incapable of functioning. Such plants classified as of first form are functionally male only. In the highly developed stamens and impotent pistils these plants may be considered as representing the extreme of maleness seen in this species. Some first form plants, however, produce seed in abundance.

SECOND FORM HERMAPHRODITE (figs. 4-6, 53).—Plants most typical of this class, as thus designated by BARTLETT (2), are especially to be distinguished from the first form by the stamens, which have shorter filaments and slender yellowish-green anthers. In most cases the anthers do not dehisce. There is no excessive development of sterile tissue in the stamens. Pollen grains are numerous, but the largest are only about half the diameter of the largest of the first form; but poor and shrunken grains appear to be no more numerous. Attempts to germinate the pollen have

been unsuccessful. Pollen grains have been removed from anthers of various ages, anthers have been artificially dried to various stages of dryness before pollen was removed, and many kinds of media have been employed. In extensive tests of pollen from 4 different plants during 2 years of bloom only one germinating grain was found, and this may have been accidentally introduced from another plant.

Accurate tests of the ability to set seed have not been made for plants that are best classed with this form. From the evidence at hand it appears that the pistils are very frequently functional, so that the plants most typical of this class are functionally female.

It will be noted later that the flowers of numerous plants which would ordinarily be classed with this form are found upon more careful examination to present somewhat decided differences indicative of various grades of maleness.

FEMALE OR PISTILLATE FORM (figs. 7-10, 58).—Plants that may be grouped in this class have flowers with rudimentary and rather reduced stamens, the tips of which only slightly or not at all protrude above the corolla. There is much variation, however, in the development of the stamens in such flowers. Frequently there is a differentiation of filaments and anthers as shown in fig. 8, and in cases even some traces of the 4 anther sacs. In other cases the stamens are more foliose, with no trace of anthers, as shown in fig. 10.

Numerous plants with this general type of stamen have what may be termed "closed" flowers; that is, the corolla lobes do not spread out and become reflexed, and when the flowers are fully developed they appear as shown in figs. 7 and 9, a condition decidedly in contrast to the reflexed corollas seen in such flowers as shown in figs. 1 and 4. Such a reduction of corolla in pistillate plants has long been recognized in gynodioecious species, and such a condition was recognized for *P. lanceolata* in the early observations made by DARWIN (7, p. 307) and LUDWIG (19, p. 322). Examination of such flowers shows that the blade portion of the petals is well developed, but that the part below the blade is shortened and often crumpled; the corolla lobes, therefore, are

not pushed up above the calyx lobes. The writer has examined at least 100 plants with this closed corolla type of flowers. In every case the stamens were scarcely or not at all exerted and were completely composed of sterile tissue.

Thus far all plants that I have seen which had completely sterile non-exserted stamens also had closed flowers; but the pistillate form as described by BARTLETT also includes plants with corollas fully developed and reflexed, and such a flower is figured by him (2, fig. 3) as illustrating a typical pistillate flower. Various plants with expanded petals and completely sterile or indehiscent stamens are potentially only females. The rudimentary development of stamens and the character of the corolla may be regarded as extreme cases of loss of maleness, and the character of the corolla may be considered as a secondary sex character associated with femaleness and appearing when maleness is most completely lacking.

From general observations of plants in the field and in a greenhouse, and from such controlled pollination as have been made, it appears that plants of this pistillate type are highly productive of seed. A few plants, however, have set no seed when exposed to favorable conditions for free cross-pollination, which suggests that the pistils of some of the pistillate plants may be impotent.

These descriptions refer to the types of flowers that characterize the 3 forms most generally recognized, and into which attempts have been made to classify all individuals. Both CORRENS (4) and BARTLETT (3), however, recognized that it was somewhat difficult to thus place all individuals observed by them. Such a difficulty has been very apparent in respect to the material studied by the writer. The variations present almost every grade of intermediates between the two extremes described, and seem to involve a series of sex intergrades or intersexes. The character of flowers may be quite uniform for a plant as a whole, or there may be a wide range of intersexuality among the different flowers of a single spike, or even among the various stamens of a single flower. Flowers typical for some of these may be described and arbitrarily numbered as follows:

INTERSEXES WITH FLOWERS UNIFORM

No. 11 (figs. 11, 12, 50).—The relative lengths of pistils and stamens in the flowers of this plant are quite as in the first form. The general appearance of the spikes in full bloom is quite similar (fig. 50), but the anthers are noticeably smaller and more narrow, and they are slightly greenish-yellow in color. Many anthers do not dehisce, and after 2 or 3 days they turn brown. A high percentage of pollen is impotent, but the size of the apparently good grains ranges quite as for the first form.

No. 13 (figs. 13, 14).—The stamens produced by this plant are somewhat smaller than those of the first form. They are slightly greenish-yellow, but are fully dehiscent. A large proportion of pollen was impotent, but a few well formed grains as large as the largest of the first form were found. Tests of pollen germination in 15-1, 15-3, and 15-5 sugar-agar media gave germination in about 3 per cent of the grains. The tubes made a feeble growth and the longest obtained measured only 0.08 mm.

No. 15 (figs. 15, 17).—Pistils of this plant are normally longer than the stamens when both are fully developed. The filaments are only slightly shorter than in the first form; the anthers are decidedly smaller, but all are white and fully dehiscent. A large proportion of the pollen is impotent, but normal grains of large size are abundant. The pistils produced by this plant were among the longest observed on any plant, except for the abnormally elongated pistils (fig. 56) which appear in plants under certain conditions.

No. 18 (figs. 18, 19).—The stamens and pistils in flowers of this plant are of nearly equal length. Nearly half of the apical portion of the stamens is composed of a sterile blade. The small anther sacs, however, are well developed and fully dehiscent. Scarcely a shriveled pollen grain was found, the grains being very uniformly of large size and a high percentage of them being viable. In this plant the amount of sterile tissue in stamens is decidedly more than that seen in nos. 1, 11-17, but there is better development of such sporogenous tissue as is formed.

No. 20 (figs. 20-22, 54).—At the time when the pistils were receptive the flowers of this plant appeared as shown in fig. 20,

with the lobes of the corolla scarcely expanding and the large anthers scarcely protruding. Several days later, when the stigmas were beginning to shrivel, the corolla was slightly expanded (fig. 21). The anthers are large, but there is marked inequality in size of the 2 pairs. The pair next to the insertion of the filament is uniformly the larger and overlaps somewhat the smaller pair, so that in face view an anther appears as in fig. 22. Dehiscence is somewhat irregular and is confined to the apex, so that few spores are shed. The anthers persist until all the flowers in a spike bloom. In old anthers the microspores are dry and shriveled, but in fresh anthers they are mostly of large size and appear to be normal; but no germination was obtained in cultures.

No. 23 (figs. 23, 24).—A plant with short crinkled filaments and extremely narrow and pale green anthers. Most anthers dehisce fully. Very few microspores are plump and have granular contents. The range of size of grains is quite as for the first form, but no germination was obtained in cultures.

No. 25 (figs. 25-27).—This plant resembles a second form hermaphrodite. The stamens, however, are decidedly shorter, the anthers are somewhat of the same shape but dehisce regularly, and the microspores range to a larger size quite as for the first form. About 20 per cent of the pollen grains tested germinated, but in all cases the tubes made only a very feeble growth.

No. 28 (figs. 28, 29).—The stamens produced by this plant have short and crinkled filaments with decidedly green anthers. The apical half of the anthers is composed of a sterile green blade, and the anther sacs are much reduced in size and are not dehiscent. At least 75 per cent of the pollen grains that are produced are of large size and are plump with granular contents. In 3 cultures of pollen removed from fully developed anthers 6 grains germinated and the best developed tube was 0.60 mm. in length.

No. 30 (figs. 30, 31).—In general appearance the stamens produced by this plant resemble those of the second form; the anthers are greenish yellow but the filaments are shorter. There is a marked peculiarity, however, in the development of anther sacs not observed thus far on any other plant. When anthers

are fully extended their appearance suggests dehiscence, but an examination at earlier stages of development shows that the 4 anther sacs develop as thin platelike and chiefly indehiscent structures, with only a few scattering thin areas of sporogenous tissue.

No. 32 (figs. 32, 33).—The stamens of this plant protrude only slightly above the throat of the corolla. The general shape of the anthers is maintained, but the anthers are wholly or nearly wholly sterile, and there are only slight irregularities on the surface suggestive of any differentiation of anther sacs.

Nos. 34 and 35.—Numerous plants are to be found having stamens with no trace of sporogenous tissue or even of anther differentiation. When such rudimentary stamens are short, they may be entirely or nearly inclosed within the corolla as previously described for certain plants classed as pistillate (figs. 9, 10). In many cases, however, the stamens are more extended and take on the character of leaves, both as to general shape and color. One of the cases best developed in this direction is illustrated in fig. 34. In fig. 35 the foliose stamens were of nearly uniform width and were much recurved.

SUMMARY.—It is difficult to arrange or classify the flowers typical of individual plants, such as described, in any fully consistent series. Various types of flower and various grades of development of stamens are to be recognized, and it is evident that as arranged in descriptions and in plates the flowers of nos. 11–32 comprise a series which presents a quite continuous gradation between such extremes as shown in figs. 1 and 7. Stamens decrease noticeably in length of filaments, in size, in shape, and in dehiscence of anthers, in the relative amount of tissue that is sporogenous, and in the total number and viability of microspores produced. Complete absence of sporogenous tissue is seen in no. 32, almost complete absence of such tissue is seen in no. 30, and indehiscence is complete in no. 28, giving plants that can function only as females. Reduction in size of anthers and of the amount of sporogenous tissue, however, does not necessarily involve also a decrease in size and viability of the spores which are produced, as is shown in no. 18. Marked differences in viability of pollen are in evidence. Rarely was any germination

observed in microspores artificially removed from indehiscent anthers. Very feeble germination was also obtained in tests of large sized grains from fully dehiscent anthers, as in no. 25. In many plants completely sterile stamens retain some suggestion of filaments and anthers in regard to the general form, but all traces of such differentiation may disappear, giving only foliose structures as shown in nos. 10, 34, and 35. In general, the various grades of development of stamens may well be regarded as indicating different grades of maleness.

In the case of all plants the flowers of which have here been described and illustrated, observations were made of flowers in numerous heads throughout at least one season of bloom. Some have been under observation for several years. At the extreme tip of the spikes in many plants there is a tendency for flowers to develop poorly; the pistils usually protrude, but the stamens are poorly formed and often flowers fail to open. This is the tendency to gynomonoecism especially emphasized by SCHULZ (24) and CORRENS (4). This tendency is evidently more marked in some plants than in others, but I am unable to make any classification on this basis. For the plants already discussed the flowers were very uniform for at least four-fifths of the spikes, as indicated in the spikes (excepting no. 55) shown in pl. 13.

GRADES OF INTERSEXUALISM

Variations in the development of stamens in the same flower or among sister flowers are frequent for many plants. In such cases there are various mixtures of different types of flowers and stamens. Some of these may be noted as follows:

No. 36 (fig. 36).—For this plant some stamens were nearly identical with those of the first form, while others were quite as in the second form. Differences in the length of stamens in a single flower were conspicuous. Some anthers failed completely to dehisce, while others dehisced fully. The greater portion of the pollen was impotent. Grains of large size were present and some of these from dehiscing anthers germinated well in cultures.

No. 37 (figs. 37, 38, 52).—As in the plant previously noted, there is much variation in the length of filaments among stamens

of the same flower. Here, however, the anthers are all quite uniform in size and shape. A rather large portion of the apex is sterile, but the anther sacs dehisce fully, and about 50 per cent of the pollen which they contain appears to be normal. In tests, however, only grains of large size germinated, and the tubes from these made only a feeble growth.

No. 39 (figs. 39, 40).—Filaments are here not only of unequal length, but all are more or less twisted, and nearly all are expanded broadly at the base of the anthers. The upper portions of the anthers are leaflike. Anther sacs vary in number and in degree of development; all 4 may be in evidence, or there may be only 2 (fig. 40), but all are more or less rudimentary and none dehisce. Nearly 30 per cent of the microspores examined were granular and of large size. Of 3 cultures, only 2 grains germinated, and the best tube obtained was 0.35 mm. in length.

No. 41 (figs. 41-43).—In the stamens of this plant the anthers are reduced to irregularly sagittate-shaped leafy structures. Such structures are often composed only of sterile tissue; in some a mere nest of spores develops, but these spores are completely imbedded in sterile tissue. In no case was more than one such nest found in a stamen. Dissection of fully mature structures revealed that the microspores were represented by shriveled cells (fig. 43).

No. 44 (figs. 44, 45).—In this plant the stamens are somewhat more leaflike than those just described. Some are completely sterile, and usually one nest, but sometimes two, of sporogenous tissue may be present in a stamen. Only a few pollen grains appear normal when dissected out.

No. 46 (figs. 46, 47, 48, 56).—A wide range of variation is seen among the stamens produced by this plant. All stamens in a flower may be completely sterile and foliose, as in fig. 46, all may have quite well developed anthers with much good pollen, or all grades between these extremes may be present; 4 stamens from a single flower are shown in fig. 48, and illustrate very well this range. Flowers growing side by side and opening at the same date exhibit wide variations and a great mixture of types. In several plants under observation this was the condition in all

spikes throughout the entire period of bloom. The general appearance of typical spikes from such plants is shown in nos. 55 and 56, but the wide range of stamen forms which are present is not clearly shown. The spikes shown in no. 56 also show the excessive growth which stigmas frequently make. It has been suggested that this occurs when pollination and fertilization have not been effected, and that successful pollination inhibits such growth. It is possible, however, that such growth is an indication of loss of femaleness. Studies are now in progress to determine especially the functional potentiality of pistils of plants for which this phenomenon is very general.

SUMMARY.—The flowers described and illustrated for plants 36-47 show that wide variations exist in the development of stamens among various flowers of a plant, or even among stamens of a single flower. The range is in some cases almost identical with the extremes seen for plants as wholes (nos. 1-35). This statement refers to the flowers produced in the lower two-thirds of the spikes. It may be noted that the range is greater for such a plant as no. 46 than for one like 36 or 37. This variation is not identical with the tendency for the last flowers of a spike to be different from earlier flowers. Here there is a marked mixture throughout the spikes.

NATURAL DISTRIBUTION

In the fields in and about the New York Botanical Garden *P. lanceolata* is so abundant that it often dominates the vegetation over a considerable area. Here plants that approach the first form are most numerous; female plants corresponding to the type described as nos. 7 and 9 are abundant; and there are thousands of plants which are in some degree intermediate between these extremes. Many plants with mixed flowers are to be found. With respect to vegetation characters and to general size and shape of spikes, extremely wide variations are everywhere in evidence.

The variations in flower forms noted by DARWIN for England, by LUDWIG and CORRENS for Germany, and by BARTLETT in the vicinity of Washington, D.C., indicate that much the same range

of variation is to be seen over a wide geographical area. Undoubtedly many of the plants classed as intermediate gynomonoecious, especially by CORRENS, present a range of variations quite identical to those here described.

The wide geographical range of this species, and especially its recent rapid spread in America, give opportunity to observe to what extent there is geographic distribution of races possessing distinctive differences in sex heteromorphism.

Discussion

The term intersexuality, as especially applied by GOLDSCHMIDT to conditions of sex in *Lymantria dispar*, can with equal adequacy be applied to such sex variations as are evident in *Plantago lanceolata*.

It must be recognized that the significance of such variations is to be sought in the conception that there may be different degrees in the expression of maleness and femaleness. Cases of intersexuality afford material for the study of stages and degrees of sexuality and sex determination.

The observations reported for *P. lanceolata* refer almost entirely to maleness. The variations in development of the stamens, with their anthers and contents, are easily and directly to be observed. Evidences of marked variations in the development and functioning of the pistils are also in evidence, and further studies of femaleness are in progress.

It is very evident that there is a wide range of variation in the degree in which maleness is expressed. Measured by the amount of sporogenous tissue, there is every degree of sexual development between the highest grade seen and complete sterility. The size of the stamen as a whole and the size and shape of its various parts exhibit a series from the normal to extremely rudimentary structures. There are two forms in which this decrease in maleness is expressed. In one the stamens are greatly reduced in size; in the other they become foliose. The foliose character is seen first in the slight enlargements of the sterile tissue at the apex of the anther, as shown in figs. 19 and 29.

It is to be recognized that the impotence of one or the other of the sex organs involved in intersexuality is to be distinguished

from sterility of the type classed as impotent (STOUT 25), which results very frequently from hybridization. In sterility of hybrids there is poor development of both sets of sex organs; stamens and pistils are both affected very uniformly, and the tendency is to give complete sterility. In intersexuality loss of sex development for one sex is not necessarily associated with similar loss in the expression of the other sex. In fact, the opposite condition is the normal one for such cases.

In *P. lanceolata* the so-called "first form" is very high in its grade of maleness, and it is in these plants apparently that seed production is noticeably low. As already stated, such plants may fail to set any seed. They have maleness well developed, but functional femaleness may be lost, although pistils are present. Likewise in the most marked cases of loss of maleness the degree of femaleness may be high, as is seen in the plants classed as females. DARWIN (7) reports that females in certain gynodioecious species (*Thymus serpyllum*, *T. vulgaris*, and *Satureia hortensis*) are much more productive of seed than the hermaphrodites, and that thus the species produces more seed than if all were hermaphrodites, a condition to which he attaches evolutionary significance in the formation and separation of the two sex forms. CORRENS (5), however, reports that the hermaphrodites of *S. hortensis* are more productive of fruit and seeds than the females.

If it is found that in *P. lanceolata* femaleness also varies in the degree of its expression, it is quite probable that increased maleness is correlated in the individual with decreased femaleness. Still it is also possible that the variations are such that both decreased maleness and femaleness may be present in the same individuals, that individuals may be intermediate for both, and that both maleness and femaleness may be well developed, giving full hermaphrodites. All these conditions, it appears, are represented in the groups of intersexes studied by GOLDSCHMIDT, BANTA, and by DAVEY and GIBSON. Such facts go far toward establishing the fundamental similarity between sex characters and every other class of structures as functional hereditary characters.

It is the tendency to a differential loss of one sex that distinguishes intersexuality from sterility (impotence) resulting from

hybridization, and from that sterility ascribed to replacement of sexual reproduction by asexual means (GATES and GOODSPEED 9), in both of which the tendency is to give a very uniform impotence of both sexes. A high degree of impotence is present in many plants regarded as pure species. JEFFREY (14, 15, 16) has recently emphasized the view that such sterility is to be considered as conclusive evidence of hybrid origin. Intersexuality, however, involves much impotence, and may very clearly develop in pure species through lability of the processes of sex determination.

At this point one may well inquire whether differences in sexuality somewhat akin to intersexuality may be present in species that are morphologically fully hermaphrodite, and in which no appreciable impotence of sex organs is in evidence. For example, DARWIN reports that plants of "the short-styled form of *Primula veris* produce more seed than the long-styled in the proportion of nearly four to three (7, p. 19), and that in *Lythrum Salicaria* (6, 7) the mid-styled form is potentially capable of higher seed production than plants or either of the other two forms. Judged on the basis of seed production, certain forms in heterostyled species appear to be more female than others. Sexuality of species as such is obviously more intense in some than in others if we are to judge by seed reproduction. Much variation in total seed production is seen among races and among individuals of a race. Such considerations raise many questions regarding the determination of potentiality of sex reproduction through production of seed, and most especially of the relations of vegetative to reproductive function. Undoubtedly much variation in maleness and femaleness exists in sex organs that are morphologically perfect. The sexual behavior of female pigeons has especially been studied by RIDDLE as an index of the degree to which femaleness is developed. He states (22, p. 341) that "females hatched from eggs laid earlier in the season are more masculine in their sex behavior than are their own full sisters hatched later in the season. And several grades of females can be thus seriated according to season of hatching."

The existence of physiological incompatibilities (STOUT 25) between sex organs that are fully formed, potentially functional,

and of simultaneous development are especially well revealed in self-fertilization of numerous species that are homomorphic hermaphrodites. Judged by ability of sex organs to function together, both femaleness and maleness of sex organs are seen in such cases to be of various grades of intensity. Such cases reveal that grades of functional or physiological sexuality may be quite independent of morphological sexuality. The striking feature of incompatibilities, however, is that sex organs which are functionless in some relations are highly functional in certain other relations. For example, it is not complete loss of femaleness, but only a loss in relation to certain degrees or grades of maleness.

The conditions that exist in *Campanula carpatica* (PELLEW 21) are of special interest in indicating that variations in the relative development of sex organs and physiological incompatibilities may both operate in a single species. PELLEW finds that there is a wide range of variations from normal hermaphrodites to females quite as I have described in *P. lanceolata*; it is also reported that nearly all hermaphrodites are self-sterile (physiological incompatibility). The "self-sterile" hermaphrodites used in the experiments set seed to cross-pollination, but the extent to which self- and cross-incompatibilities may be operating among hermaphrodites and in crosses of hermaphrodites with females was not determined, and the studies do not reveal whether or not some plants classed as hermaphrodites may be impotent as to femaleness.

The inheritance of various grades of intersexes in *P. lanceolata* is a problem under investigation, and a discussion of the researches (CORRENS, BARTLETT, GOLDSCHMIDT, RIDDLE, PELLEW, etc.) bearing on this question therefore will not be made here.

It is quite clear that sex differentiation is to be considered as morphological and as physiological. Physiologically the essential and only index of sex in cells is the capacity for their fusion which culminates in the expression of that function by sex cells. It is in decided contrast to that property of asexuality which is seen in cell division and cell growth.

Morphological sexuality consists purely and solely of adaptations to facilitate the bringing into juxtaposition cells that are

capable of fusion when juxtaposed. It may consist of (1) the more or less immediate modification of physical structure of the cells (in spermatogenesis and oogenesis) that are to fuse, and (2) of modifications of organs associated with the development of sex cells, either in the sporophyte or the gametophyte, or both. All of these latter are in reality secondary sex characters; true primary sex characters are to be considered as belonging to the cells that fuse, a view clearly stated by STRASBURGER (26).

The relationship between morphological and physiological sex differentiation is well shown in the flowering plants. We may take a hermaphrodite with perfect flowers as a type. Primarily such a plant is a spore-producing individual; it is a sporophyte in which heterospory is in evidence. The stamens bear microspores, the pistils bear macrospores. These spores are asexual in that they are not able to fuse. They are sexual, however, to the extent that sex is here already determined. Anatomical expression of maleness and femaleness here appears in sporophytic structures, and the particular sex of the future generations of cells in asexual descent is predetermined until the next fusion of sex cells or the development of a sporophyte through apogamy. The pollen grains grow into microgametophytes producing male sex cells or sperms. The macrospores grow into the megagametophytes which produce the eggs. The alternation of generations is marked; the one is sporophytic; the other is gametophytic. But maleness can be traced back through the pollen tube, through pollen, beyond reduction divisions, to the beginning of somatic differentiation of stamens. Likewise femaleness can be traced to the beginning of the organogenesis of the pistil. These facts certainly justify the application of the terms male and female to structures that in their morphology are sporophytic. This view has frequently been criticized by those who emphasize the morphology of the alternation of generations (MACMILLAN 20). Furthermore, it is to be noted that in the greater number of animals the gametophytic generation is omitted or perhaps to be considered as reduced to a single cell generation, and that here the conditions of maleness and femaleness are most essentially properties of individuals and structures that are wholly sporophytic.

It is clear that in the higher flowering plants maleness and femaleness are two series of morphological steps beginning in the development of stamens and pistils from cells of the closest somatic lineage. Any diploid or haploid nuclear organization can become either male or female according to whether its cell lineage leads through stamens or pistils. In this sense maleness and femaleness are acquired; they are conditions imposed upon cell organization rather than existing as separate inherent conditions; they begin in somatic differentiation that is fundamentally on the same basis as differentiation of stems, leaves, and sterile floral organs. Potentially maleness and femaleness (either morphological or physiological) reside in every cell of the sporophyte. The reduction divisions preceding the gametophytic divisions give the same range of nuclear organization to both kinds of spores.

It is such conditions, emphasized by the wide occurrence of hermaphrodites, that compel us to state the problems of sex determination in such questions as the following:

1. What physiological and chemical processes operate when sex differentiation appears and is initiated morphologically among organs which develop side by side from cells of the same somatic lineage?

2. Should we not regard dioecism as the suppression of maleness or femaleness in an individual as a whole (either in sporophytic or in gametophytic generations, or in both)?

We may note that intersexuality completely fills the gap between hermaphroditism and dioecism. In this respect the conditions in plants fully agree and supplement those reported in animals. Viewing all the evidence, we may at the present time make the following conclusions, which are in general harmony with the facts and the conclusions of GOLDSCHMIDT, BANTA, RIDDLE, and LILLIE: (1) Fundamentally maleness and femaleness reside in all somatic cells of all sporophytic individuals. (2) Maleness and femaleness are quantitative differentiations; there are all grades of intersexes. Maleness and femaleness are relative; there are all grades of compatibilities. (3) Sex determination, at least in hermaphrodites, is fundamentally a phenomenon of somatic differentiation that is ultimately associated with

processes of growth, development, and interaction of tissues, and subject to modification or even complete determination by them.

The older conception of mystical properties of maleness and femaleness have given place to what are fundamentally metabolic theories of sex determination. The principal points of difference in the large number of theories, thus to be grouped, lie in questions regarding (1) time of determination, (2) whether the two sexes are two contrasted conditions or simply phases of the same general property, (3) to what extent sex development in the individual is an evolution or an epigenesis, and (4) to what extent a physical basis can be related to differences in the amount of chromatin present.

To DARWIN and many of his contemporaries the evolutionary and adaptational significance of variations in sex were points of principal interest. That such variations fundamentally involve physiological processes operating in the organism was of course recognized. The increased femaleness seen in females of certain gynodioecious species was considered by DARWIN as involving the principle of compensation; with decreased expenditure of energy in development of male organs there was a greater supply for development and function of female organs. The doctrine of conservation in expenditure for useless organs was likewise applied to the tendency to gynomonoecism as seen in such a species as *P. lanceolata* (LUDWIG 19); the stamens in the uppermost flowers of a spike tend to be useless, and this was supposed to induce their elimination. The tendency to poor development of flowers at the tips of spikes, however, may be purely the result of food supply being diverted for use of lower flowers, and as such may be on quite a different physiological basis from the condition that makes an individual only female. The intimate association of many proterogynous flowers in a spike, however, may well give opportunity for changes in metabolic processes (RIDDLE) or influence of hormones (LILLIE).

A very interesting and suggestive conception which has frequently been proposed is embodied in the view that maleness is a "katabolic habit" of body (we may now add *of an organ*) induced by preponderance of waste over repair, and that femaleness is an

"anabolic habit" induced by conditions favoring constructive processes (GEDDES and THOMSON 10).

The physical basis for different metabolic activity is to be sought in qualitative or quantitative differences; the same kind of substance may be involved quantitatively or different substances may be involved either qualitatively or quantitatively.

The recent theory of the sex chromosome is in one aspect a metabolic theory in which different amounts of chromatin material in the nucleus may be considered as affording a physical basis for quantitative and perhaps qualitative metabolic differences. The theory fails as a broad biological law in not applying to the conditions of hermaphroditism as already discussed, and also in assuming that in dioecious species there is a determination of sex at the time of fertilization that is exclusive for the zygote. As intersexuality reveals, sex in zygotes of dioecious species is not necessarily irreversible (see especially RIDDLE and LILLIE); and experimental work has shown (see especially RIDDLE) that the distribution of sexes among the offspring may be controlled in a measure which breaks up the chromosomal correlation.

Most noteworthy of the more recent experimental data bearing on the chemical nature of sex determination are the results of RIDDLE. He has shown that in the pigeon "the male sex is an expression of metabolism at a higher level, the female sex of metabolism at a lower or more conservative level" (22, p. 322). The chemical nature of the eggs produced by a single female mated with a male is found to be subject to change according to whether egg production is forced or otherwise, and sex can thus be controlled. The physical basis for differences in metabolic activity is to be found in changes in the chemical organization and relations of the food substances. That such changes can readily occur is quite in harmony with well known facts as to the chemical differences in metabolic substances produced by an organ under different conditions. In the case of sex control in the pigeons it appears that it is not the amount of one or more kinds of food substances, but the different chemical nature of them, induced by the condition of the mother, that leads to differences in metabolism which determine the sex of the offspring.

The development of perfect flowers in hermaphrodites shows that male and female organs may originate side by side. That stamens and pistils exhibit differences in nutritive and metabolic activities is obvious, most marked of which perhaps is the temporary nature of the stamens and the more permanent and vegetative nature of the ovary portion of the pistil. The life processes of the two develop along somewhat different lines, as the structure and physiology of the respective spores, gametophytes, and sex cells fully indicate. Such organic specificity is well known frequently to involve specific differences in chemical organization. This, however, is not indicative that the essential nature of fertilization processes is dependent on such differences.

There seems to be no exception to the rule that in perfect flowers the male organs constitute an outer and lower whorl, the primary anlagen of which are laid down slightly ahead of those for the female. Such a general mode of development it would seem must have special significance in respect to sex differentiation. Such conditions, however, are adaptive both to immediate and to more remote function of the parts involved. When conditions in monoecious forms are reviewed it is to be noted that when grouped in spikes and catkins the staminate flowers are as a rule about the pistillate, either when both are in a same catkin or when they are in different catkins. Here, however, direct adaptations for facilitating pollination are in evidence.

The phenomena of intersexuality in plants and animals indicate clearly that neither hermaphroditism nor dioecism are fixed conditions for species or for individuals as such. Maleness and femaleness are subject to much lability; they are even reversible; the physical and chemical substances involved are subject to modification in ontogeny. The factors in sex determination for the individual as a whole or for individual sex organs are highly variable. Such conditions give support to a metabolic and epigenetic theory of sex in so far as the nature of sex is revealed in the morphological differentiation of sex organs.

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